

# Organic Phosphorus Composition and Potential Bioavailability in Semi-Arid Arable Soils of the Western United States

Benjamin L. Turner,\* Barbara J. Cade-Menun, and Dale T. Westermann

## ABSTRACT

The organic P composition of semi-arid arable soils is largely unknown, but such information is fundamental to understanding P dynamics in irrigated agriculture. We used solution  $^{31}\text{P}$  nuclear magnetic resonance (NMR) spectroscopy and phosphatase hydrolysis to characterize organic P in semi-arid arable soils from the western USA (organic C 2.0–30.7 g C kg $^{-1}$  soil, clay 2–48%, pH 5.2–8.2,  $\text{CaCO}_3$  <1–480 g kg $^{-1}$  soil). Total P concentrations ranged from 220 to 1210 mg P kg $^{-1}$  soil, of which between 12 and 45% was extracted with NaOH–EDTA. Inorganic orthophosphate was the dominant P compound, but concentrations determined by solution  $^{31}\text{P}$  NMR spectroscopy were consistently greater than those determined by molybdate colorimetry. Concentrations of organic P were relatively small, and were dominated by orthophosphate monoesters (11–130 mg P kg $^{-1}$  soil), with smaller concentrations of orthophosphate diesters (0–7 mg P kg $^{-1}$  soil). Pyrophosphate was present in almost all soils at concentrations up to 14 mg P kg $^{-1}$  soil. Bicarbonate-extractable organic P ranged from 1.7 to 22.8 mg P kg $^{-1}$  soil, of which between 37 and 87% was hydrolyzed by phosphatase enzymes, suggesting its bioavailability. Soil organic P concentrations were positively correlated with mean annual precipitation, organic C, clay, and oxalate-extractable metals (Al, Fe, Mn), and negatively correlated with mean annual temperature and soil pH. However,  $\text{CaCO}_3$  concentrations were not significantly correlated with any soil property. These results indicate that equilibrium levels of organic P in semi-arid arable soils are controlled by a balance between the physical protection offered by the soil matrix and the suitability of the environment for biological productivity.

INFORMATION on the chemical composition of soil organic P is fundamental to understanding plant nutrition and soil biogeochemical cycles. However, organic P remains poorly understood, despite constituting a large proportion of the total P in many soils, and providing a source of P for plant uptake (Magid et al., 1996). In particular, little information exists on the chemical composition of organic P in low organic matter calcareous soils, such as those common in the drier regions of the western USA. The semi-arid climate of the western USA means that most agricultural soils are irrigated, and such soils are agronomically important because irrigated agriculture produces nearly 40% of the total U.S. crop value from only 15% of the total cropped land (Bajwa et al., 1992). Therefore, information on the composition of organic P and its potential contribution to plant nutrition in these soils must be better understood. Furthermore, current interest in the transfer of soil organic P compounds to watercourses and their impact on water quality (Haygarth and Jarvis, 1999) reinforces

the need to understand the nature of organic P in these soils.

Soil organic P determination has traditionally been hampered by difficulties with the extraction, separation and detection of these often recalcitrant compounds, but analysis by solution  $^{31}\text{P}$  NMR spectroscopy obviates many of these problems. When coupled with the single-step NaOH–EDTA extraction procedure (Bowman and Moir, 1993) more organic P can be characterized than by extraction with NaOH alone (Cade-Menun and Preston, 1996). Indeed, more than 90% of the total P in high organic matter soils has been characterized by NaOH–EDTA extraction and solution  $^{31}\text{P}$  NMR spectroscopy (Dai et al., 1996; Cade-Menun et al., 2000). Solution  $^{31}\text{P}$  NMR spectroscopy is less suitable for the analysis of water or bicarbonate extracts, because the relatively high detection limits confound analysis of samples with low P concentrations (Nanny and Minear, 1997). Despite this, the organic P in these types of extracts can be characterized by its susceptibility to hydrolysis by phosphatase enzymes (Shand and Smith, 1997; Turner et al., 2002a). This sensitive technique not only provides structural information on the extracted organic P, but also indicates its potential biological availability.

Our objectives were to determine the composition and potential bioavailability of organic P in a range of low organic matter, mainly calcareous, arable soils of the semi-arid western USA, and to investigate how these were related to climate and soil properties.

## MATERIALS AND METHODS

### Soil Sampling, Preparation, and Determination of Basic Properties

Eighteen soils were sampled to 30-cm depth from locations around the western USA during 2000 (Table 1). Most soils were under irrigated arable cropping, although the Wahpeton soil (No. 18) (soil descriptions are given in Table 1) was not irrigated and the native Portneuf (No. 14) was under non-irrigated sagebrush (*Artemisia tridentata* Nutt.). The sampling sites were semi-arid, with hot dry summers and cool moist winters. Mean annual temperatures ranged from 5.0°C at Fargo, ND, to 13.8°C at Amarillo, TX. Mean annual precipitation ranged from 209 mm at Othello, WA, to 547 mm at Pullman, WA.

The soils were air-dried (30°C), sieved (<2 mm), and stored at ambient laboratory temperature before analysis. Soil textural information and the concentrations of organic C and  $\text{CaCO}_3$  were determined by standard methods (Allison and Moodie, 1965; Day, 1965; Nelson and Sommers, 1982). Soil pH was determined on a saturated paste using deionized water (1:1 ratio). Oxalate-extractable Al, Fe, and Mn were determined by extraction with ammonium oxalate/oxalic acid (pH 3.0) for 2 h, followed by detection using inductively coupled plasma atomic-emission spectroscopy (ICP–AES) (Schoumans, 2000).

B.L. Turner and D.T. Westermann, USDA–ARS, Northwest Irrigation and Soils Research Lab., 3793 N. 3600 E., Kimberly, ID 83341; B.J. Cade-Menun, Dep. of Geological and Environmental Sciences, Stanford Univ., Stanford, CA 94305. Received 1 July 2002. \*Corresponding author (bturner@nwisrl.ars.usda.gov).

**Table 1.** Description, location, and climate information for the 18 western U.S. soils. The soils are ranked in order of their organic C concentrations (see Table 3). Conventionally managed soils (conv) received only inorganic fertilizer, whilst manured soils received a single application of dairy manure several years before sampling.

Soil series	Location	Description	Mean annual temperature	Mean annual precipitation
			°C	mm
1. Taunton	Buhl, ID	Coarse-loamy, mixed, superactive, mesic Xeric Haplodurids	9.1	243
2. Olton	Amarillo, TX	Fine, mixed, superactive, thermic Aridic Paleustolls	13.8	497
3. Declo	Aberdeen, ID	Coarse-loamy, mixed, superactive, mesic Xeric Haplocalcids	7.2	224
4. Warden	Othello, WA	Coarse-silty, mixed, superactive, mesic Xeric Haplocambids	10.1	209
5. Amarillo	Amarillo, TX	Fine-loamy, mixed, superactive, thermic Aridic Paleustolls	13.8	497
6. Portneuf (conv subsoil)†	Kimberly, ID	Coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcids	8.7	275
7. Greenleaf	Parma, ID	Fine-silty, mixed, superactive, mesic Xeric Calciargids	10.2	260
8. Williams	Sidney, MT	Fine-loamy, mixed, superactive, frigid Typic Argistolls	5.9	354
9. Portneuf (manured subsoil)†	Kimberly, ID	Coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcids	8.7	275
10. Portneuf (conv)	Kimberly, ID	Coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcids	8.7	275
11. Roza	Filer, ID	Fine, smectitic, mesic Xerertic Haplocambids	8.9	259
12. Portneuf (manured)	Kimberly, ID	Coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcids	8.7	275
13. Millville	Logan, UT	Coarse-silty, carbonatic, mesic Typic Haploxerolls	8.6	420
14. Portneuf (native)	Murtaugh, ID	Coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcids	8.7	275
15. Brinegar	Fairfield, ID	Fine-loamy, mixed, superactive, frigid Pachic Ultic Argixerolls	5.3	391
16. Palouse	Pullman, WA	Fine-silty, mixed, superactive, mesic Pachic Ultic Haploxerolls	8.3	547
17. Labenzo	Rexburg, ID	Fine-loamy over sandy or sandy-skeletal, mixed, superactive, frigid Fluventic Haploxerolls	6.4	348
18. Wahpeton	Fargo, ND	Fine, smectitic, frigid Typic Hapluderts	5.0	494

† Sampled from experimental plots from which topsoil was removed to simulate the effects of soil erosion exposing highly calcareous subsoil (Robbins et al., 1999).

### Methodology for Characterizing Soil Phosphorus

Soil P was characterized using three procedures (Fig. 1):

1. Determination of total soil P fractions based on digestion, ignition, and acid-extraction;
2. Characterization of NaOH-EDTA extractable P by molybdate colorimetry and solution  $^{31}\text{P}$  NMR spectroscopy;
3. Characterization of bicarbonate-extractable P by molybdate colorimetry and phosphatase hydrolysis.

### Determination of Total Soil Phosphorus Fractions

Total soil P was determined by NaOH fusion (Smith and Bain, 1982). Total inorganic and organic P were determined by the ignition method (Saunders and Williams, 1955). Samples of ignited (550°C, 2 h) and unignited soils were extracted for 2 h with 1 M  $\text{H}_2\text{SO}_4$ . Organic P was calculated as the difference between inorganic P in the ignited and unignited samples, while residual P was calculated as the difference between total soil P and inorganic P in the ignited sample.

### NaOH-EDTA Extraction and Solution $^{31}\text{P}$ NMR Spectroscopy

Phosphorus was extracted by shaking 5 g of soil with 100 mL of a solution containing 0.25 M NaOH and 0.05 M EDTA for 16 h at 20°C (Cade-Menun and Preston, 1996). This single-step extraction of organic P is comparable with other alkaline and acid extraction procedures for calcareous soils (Bowman and Moir, 1993). We did not use an acid pre-extraction, because this also extracted some organic P. The NaOH-EDTA extracts were centrifuged at  $10\,000 \times g$  for 30 min, rapidly

frozen at  $-80^\circ\text{C}$ , and then freeze-dried over several days. Inorganic P was determined colorimetrically in diluted extracts (1:100) by molybdate reaction with P detection at 880 nm (Murphy and Riley, 1962). The extracts must be diluted at least 20-fold to avoid interference by EDTA in the molybdate reaction. Total P was determined by ICP-AES and organic P was calculated as the difference between total and inorganic P. The inorganic P fraction is mainly orthophosphate, but can include acid-labile organic and condensed P compounds (Dick and Tabatabai, 1977), while the organic P fraction also includes inorganic polyphosphates (Shand et al., 2000). However, we use the terms inorganic and organic P for clarity. All results are means of three replicate analyses with standard errors (not shown) less than  $\pm 5\%$  of the mean value.

Freeze-dried NaOH-EDTA extracts (approximately 1 g) were redissolved in 0.4 mL of 10 M NaOH and 2.6 mL of  $\text{D}_2\text{O}$  and allowed to stand for 30 min with occasional vortexing. Samples were then centrifuged for 20 min at approximately  $1500 \times g$ , transferred to 10-mm diam. NMR tubes and stored at  $4^\circ\text{C}$  before analysis within 24 h. Solution  $^{31}\text{P}$  NMR spectra were obtained using a GE Omega 500 MHz spectrometer equipped with a 10-mm broadband probe (General Electric, Fremont, CA). We used a  $90^\circ$  pulse, 0.68-s acquisition time, and 4.32-s pulse delay. Temperature was regulated at  $25^\circ\text{C}$  but it is now recommended that temperature be regulated at  $20^\circ\text{C}$  for solution  $^{31}\text{P}$  NMR spectroscopy of soil extracts (Turner et al., 2003). An equal number of scans (8000) was collected for each sample and compounds were identified by their chemical shifts (ppm) relative to an external  $\text{H}_3\text{PO}_4$  standard. General functional classes of P compounds were as follows: inorganic orthophosphate at approximately 6.1 ppm,

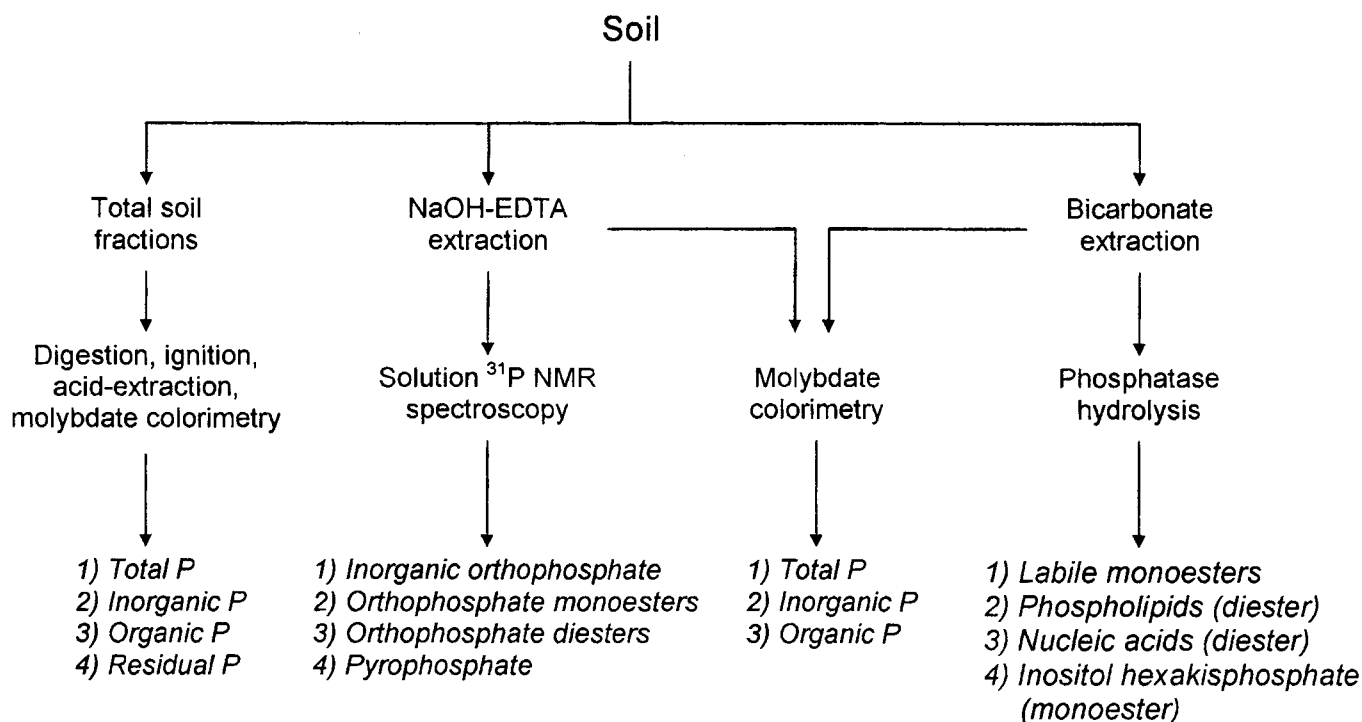


Fig. 1. The fractionation scheme used to characterize soil inorganic and organic P.

orthophosphate monoesters between 3 and 6 ppm, orthophosphate diesters between  $-0.5$  and  $2.0$  ppm, pyrophosphate at approximately  $-4.5$  ppm (Turner et al., 2003). The limit of detection using solution  $^{31}\text{P}$  NMR is difficult to quantify, but depends on the P concentration of the freeze-dried extracts and the number of scans acquired. For our study we estimate the limit of detection to be approximately  $1 \text{ mg P kg}^{-1}$  soil.

#### Bicarbonate Extraction and Analysis by Phosphatase Hydrolysis

Bicarbonate-extractable P was determined by shaking  $2.5 \text{ g}$  of soil with  $50 \text{ mL}$  of  $0.5 \text{ M NaHCO}_3$  (adjusted to pH 8.5 with dilute NaOH) for 30 min (Olsen et al., 1954). Each sample was filtered sequentially through a Whatman No. 42 filter paper (Whatman Ltd., Maidstone, UK) and a  $0.2\text{-}\mu\text{m}$  cellulose acetate syringe filter (Nalgene, Rochester, NY). One-milliliter aliquots were pre-acidified with  $0.1 \text{ mL}$  of  $3 \text{ M H}_2\text{SO}_4$  (to remove carbonates) and diluted to  $5 \text{ mL}$  with deionized water. Inorganic P was then determined by adding  $1 \text{ mL}$  of molybdate reagent and measuring the absorbance after 12 min at  $880 \text{ nm}$ . Molybdate-reactive P in bicarbonate extracts includes only inorganic orthophosphate (Coventry et al., 2001). Total P in the extracts was determined by the same procedure fol-

lowing acid-persulphate digestion. Briefly, samples ( $1 \text{ mL}$ ) were acidified by adding  $0.15 \text{ mL}$  of  $3 \text{ M H}_2\text{SO}_4$  and digested with  $3.85 \text{ mL}$  of  $26 \text{ mM K}_2\text{S}_2\text{O}_8$  ( $20 \text{ mM}$  final concentration) at  $120^\circ\text{C}$  and  $100 \text{ kPa}$  for 45 min. Organic P was calculated as the difference between total and inorganic P. Each soil was extracted three times.

For 11 of the 18 soils, bicarbonate extracts were analyzed by phosphatase hydrolysis using the methodology of Turner et al. (2002a), with modifications for bicarbonate extracts, including stronger buffer concentration and the inclusion of a phospholipase. Samples ( $1 \text{ mL}$ ) were pre-acidified to remove carbonate by adding  $0.1 \text{ mL}$  of  $3 \text{ M H}_2\text{SO}_4$ , then neutralized by adding  $0.12 \text{ mL}$  of  $1 \text{ M NaOH}$ . After adding  $1 \text{ mL}$  of  $25 \text{ mM NaN}_3$  to prevent microbial activity, enzyme hydrolysis was initiated by adding  $0.25 \text{ mL}$  of  $2 \text{ M}$  buffer ( $0.1 \text{ M}$  final concentration) containing enzyme and  $2 \text{ mM MgCl}_2$  (see Table 2 for enzyme preparations). Samples were diluted to  $5 \text{ mL}$  with deionized water and incubated for 16 h overnight at  $37^\circ\text{C}$  in a shaking water bath. After incubation, the enzyme reactions were terminated and inorganic P determined by adding  $1\text{-mL}$  molybdate reagent as described previously. Phosphodiesterase and phytase caused slight interference with the molybdate reaction; so separate calibration curves were prepared from orthophosphate standards containing the enzymes.

Table 2. The enzyme preparations used to determine phosphatase hydrolyzable P.

Enzyme	Type	Source	Specified activity	Sigma No.	Buffer	Activity of preparation unit(s) $\text{mL}^{-1}$
Alkaline phosphatase (EC 3.1.3.2)	Type V-IIS	Bovine intestinal mucosa	2980 units $\text{mg}^{-1}$ protein (10 $\text{mg}$ protein $\text{mL}^{-1}$ )	P 5521	2 M Tris-HCl, pH 8.0	1
Phospholipase C† (EC 3.1.4.3)			1076 units $\text{mg}^{-1}$ protein (0.9 $\text{mg}$ protein $\text{mL}^{-1}$ )	P 7147	2 M Tris-HCl, pH 8.0	1
Phosphodiesterase† (EC 3.1.4.1)	Phosphodiesterase 1, Type IV	<i>Crotalus atrox</i> venom	0.031 units $\text{mg}^{-1}$ solid	P 4506	2 M Tris-HCl, pH 8.0	0.016
Phytase (EC 3.1.3.8)	myo-inositol hexakisphosphate 3-phosphohydrolase	<i>Aspergillus ficuum</i>	3.5 units $\text{mg}^{-1}$ solid	P 9792	2 M Glycine-HCl, pH 2.5	0.5

† The preparation used in the phosphatase hydrolyzable P assays included alkaline phosphomonoesterase.

Functional classes of organic P compounds were calculated as follows: (i) labile monoester P (hydrolyzed by alkaline phosphatase); (ii) phospholipids (the difference between the P released by phospholipase + alkaline phosphatase and alkaline phosphatase alone); (iii) nucleic acids (the difference between the P released by phosphodiesterase + alkaline phosphatase and alkaline phosphatase alone); (iv) Inositol hexakisphosphate (the difference between the P released by phytase and all other treatments). These were grouped into total monoester P (labile monoester P and inositol hexakisphosphate) and orthophosphate diesters (phospholipids and nucleic acids).

### Statistical Analysis

Concentrations are expressed on the basis of oven-dried soil (105°C). A correlation matrix ( $r$  values) was calculated to investigate relationships between soil properties and P compounds, which were then investigated visually by plotting on an  $x$ - $y$  scatter graph. Regression models were calculated using least squares linear regression. All analysis was performed using standard procedures in Microsoft Excel (Microsoft Inc., Redmond, WA).

## RESULTS

### Soil Properties

Soil physical and chemical properties are presented in Table 3. The soils are ranked in ascending order of organic C concentrations, which ranged between 1.98 and 30.69 g C kg<sup>-1</sup> soil. Soil pH ranged between 5.2 and 8.2, with most soils being greater than pH 6.5. Clay contents ranged between 2 and 48%, and most soils contained significant concentrations of CaCO<sub>3</sub> (<1–480 g kg<sup>-1</sup> soil). Concentrations of oxalate-extractable metals (g kg<sup>-1</sup> soil) were: Al = 0.26 to 1.21, Fe = 0.20 to 2.36, Mn = 0.11 to 1.07.

### Total and Extractable Phosphorus Concentrations

Total P concentrations ranged between 220 and 1210 mg P kg<sup>-1</sup> soil, of which inorganic P constituted between 27 and 76% (Table 4). Organic P concentra-

tions determined by the ignition method were between 18 and 280 mg P kg<sup>-1</sup> soil (3–36% total P). Using organic P determined by the ignition method, the organic C/organic P ratios ranged between 37 and 131 (mean 75).

Between 12 and 45% of the total soil P was recovered by extraction with NaOH-EDTA (Table 4). Of this, between 21 and 178 mg P kg<sup>-1</sup> soil was organic P, representing recoveries of between 23 and 118% (mean 63%) of the total organic P determined by the ignition method. A smaller proportion of the inorganic P was recovered (12–95% total inorganic P, mean 31%). Using organic P determined by NaOH-EDTA extraction and molybdate colorimetry, the organic C/organic P ratios ranged between 50 and 198 (mean 125).

Extraction with bicarbonate recovered between 1.6 and 13.4% of the total soil P (Table 4). Of this, between 6.5 and 106 mg P kg<sup>-1</sup> soil was inorganic P and between 1.7 and 22.8 mg P kg<sup>-1</sup> soil was organic P. The organic P concentrations represented recoveries of between 1.5 and 12.0% of the total organic P determined by ignition.

### Organic Phosphorus Composition of NaOH-EDTA Extracts Determined by Solution <sup>31</sup>P NMR Spectroscopy

The NaOH-EDTA extracts contained inorganic orthophosphate, orthophosphate monoesters, orthophosphate diesters, and pyrophosphate (Table 5, Fig. 2). Phosphonates and polyphosphates were not detected in any samples. Inorganic orthophosphate dominated the extracts of all soils, although concentrations determined by solution <sup>31</sup>P NMR spectroscopy were consistently greater than those determined by molybdate colorimetry (Fig. 3). Orthophosphate monoesters were the major group of organic P compounds, constituting between 11 and 130 mg P kg<sup>-1</sup> soil (10–44% extracted P) (Table 5). Orthophosphate diesters were not detected by solution <sup>31</sup>P NMR spectroscopy in 11 of the soils, but concentrations in the remaining soils ranged between 1.4 and 7.0 mg P kg<sup>-1</sup> soil (0.7–2.6% extracted P). Total organic P concentrations detected by NaOH-EDTA extraction and solution <sup>31</sup>P NMR spectroscopy were, therefore,

Table 3. Physical and chemical properties of the 18 western U.S. soils, ranked in order of their organic C concentrations.

Soil type	Textural class	Texture			pH	Organic C	CaCO <sub>3</sub>	Oxalate-extraction		
		Clay	Silt	Sand				Al	Fe	Mn
		%						g kg <sup>-1</sup> soil		
1. Taunton	Sand	2	8	90	8.2	1.98	7	0.26	0.36	0.11
2. Olton	Clay loam	17	20	63	7.8	3.37	1	0.46	0.22	0.14
3. Declo	Loam	15	27	58	7.9	4.51	99	0.44	0.32	0.13
4. Warden	Very fine sandy loam	10	41	49	8.1	5.35	22	0.80	1.30	0.27
5. Amarillo	Fine sandy loam	14	16	70	7.7	5.35	1	0.47	0.31	0.11
6. Portneuf (conv subsoil)	Silt loam	15	68	17	8.1	5.49	234	0.40	0.20	0.14
7. Greenleaf	Silt loam	23	49	28	7.7	5.88	29	1.09	1.70	0.34
8. Williams	Loam	14	21	65	7.1	8.02	4	0.43	0.60	0.29
9. Portneuf (manured subsoil)	Silt loam	20	64	16	8.1	8.12	234	0.46	0.27	0.14
10. Portneuf (conv)	Silt loam	26	56	17	7.9	8.23	47	0.75	0.65	0.47
11. Roza	Clay loam	22	50	28	6.9	8.81	2	0.70	0.82	0.44
12. Portneuf (manured)	Silt loam	23	57	19	8.0	9.11	106	0.62	0.52	0.34
13. Millville	Silt loam	15	47	38	8.1	10.39	480	0.74	0.63	0.12
14. Portneuf (native)	Silt loam	15	62	23	6.7	13.42	3	0.58	0.92	0.53
15. Brinegar	Loam	19	32	49	5.6	13.46	2	0.57	1.56	0.20
16. Palouse	Silt loam	24	58	18	5.2	18.23	<1	1.21	2.36	0.40
17. Labenzo	Silt loam	19	41	40	7.6	25.34	48	0.74	0.86	0.23
18. Wahpeton	Silty clay	48	37	15	6.4	30.69	6	0.75	2.06	1.07

Table 4. Inorganic and organic P concentrations in the whole soil, NaOH-EDTA extracts, and bicarbonate extracts of the 18 western U.S. soils. Organic C/organic P ratios are calculated from organic P determined by ignition and by NaOH-EDTA extraction.

Soil type	Whole soil†				NaOH-EDTA extractable P‡				Bicarbonate extractable P‡				Organic C-to-P ratios	
	Total P	Inorganic P	Organic P	Residual P	Total P	Inorganic P	Organic P	Total P	Inorganic P	Organic P	Organic P	Ignition	NaOH-EDTA	
mg P kg <sup>-1</sup> soil														
1. Taunton	568	424 (75)	18 (3)	126 (22)	103 (18)	82 (19)	21 (118)	14.0 (2.5)	12.3 (2.9)	1.7 (9.8)	114	97		
2. Olton	220	60 (27)	44 (20)	116 (53)	73 (33)	44 (74)	29 (66)	19.1 (8.7)	17.3 (29.0)	1.8 (4.1)	78	118		
3. Declo	827	568 (69)	119 (14)	140 (17)	221 (27)	131 (23)	90 (75)	21.7 (2.6)	19.2 (3.4)	2.5 (2.1)	38	50		
4. Warden	1210	890 (74)	67 (6)	253 (21)	175 (14)	112 (13)	63 (94)	19.3 (1.6)	15.6 (1.8)	3.7 (5.6)	80	85		
5. Amarillo	251	74 (29)	68 (27)	109 (43)	112 (45)	70 (95)	42 (62)	33.7 (13.4)	29.6 (40.2)	4.1 (6.0)	78	126		
6. Portneuf (conv subsoil)	970	701 (72)	147 (15)	122 (13)	116 (12)	82 (12)	34 (23)	32.3 (3.3)	30.1 (4.3)	2.2 (1.5)	37	163		
7. Greenleaf	1058	792 (75)	88 (8)	178 (17)	205 (19)	142 (18)	63 (72)	17.7 (1.7)	13.4 (1.7)	4.3 (4.9)	67	93		
8. Williams	439	182 (42)	119 (27)	138 (31)	128 (29)	54 (30)	74 (62)	19.4 (4.4)	15.1 (8.3)	4.3 (3.6)	67	108		
9. Portneuf (manured subsoil)	1135	859 (76)	172 (15)	104 (9)	255 (22)	200 (23)	55 (32)	110.5 (9.7)	105.8 (12.3)	4.7 (2.8)	47	148		
10. Portneuf (conv)	966	641 (66)	193 (20)	132 (14)	217 (22)	138 (22)	79 (41)	22.2 (2.3)	18.5 (2.9)	3.7 (1.9)	43	104		
11. Roza	729	421 (58)	91 (12)	217 (30)	154 (21)	93 (22)	61 (67)	27.6 (3.8)	22.3 (5.3)	5.3 (5.8)	97	145		
12. Portneuf (manured)	1070	764 (71)	158 (15)	148 (14)	286 (27)	194 (25)	92 (58)	57.7 (5.4)	54.4 (7.1)	3.3 (2.1)	58	100		
13. Millville	762	452 (59)	189 (25)	121 (16)	224 (29)	135 (30)	89 (47)	11.9 (1.6)	6.5 (1.4)	5.4 (2.9)	55	116		
14. Portneuf (native)	890	564 (63)	189 (21)	137 (15)	192 (22)	124 (22)	68 (36)	39.2 (4.4)	31.4 (5.6)	7.8 (4.1)	71	198		
15. Brinegar	626	312 (50)	130 (21)	184 (29)	214 (34)	123 (39)	91 (71)	40.2 (6.4)	25.2 (8.1)	15.0 (11.6)	104	148		
16. Palouse	1000	512 (51)	189 (19)	299 (30)	284 (28)	140 (27)	144 (76)	53.3 (5.3)	30.5 (6.0)	22.8 (12.0)	96	127		
17. Labenzo	1000	603 (60)	280 (28)	117 (12)	323 (32)	145 (24)	178 (63)	38.4 (3.8)	27.4 (4.5)	11.0 (3.9)	90	142		
18. Wahpeton	657	235 (36)	235 (36)	187 (29)	272 (41)	107 (46)	165 (70)	31.9 (4.9)	22.1 (9.4)	9.8 (4.2)	131	186		

† Values in parentheses are the proportion (%) of the total soil P.

‡ Values in parentheses are the proportion (%) of the respective whole soil P fraction.

between 11 and 132 mg P kg<sup>-1</sup> soil (10–47% extracted P). Pyrophosphate was detected in all but four soils (although there were traces in two of those) at concentrations between 1.1 and 13.5 mg P kg<sup>-1</sup> soil (0.5–4.3% extracted P). A trace of a compound with a chemical shift upfield from inorganic orthophosphate at approximately 6.5 ppm was identified in one soil (Greenleaf), which may represent an aromatic orthophosphate diester (Turner et al., 2003).

### Phosphatase Hydrolysis of Bicarbonate-Extractable Organic Phosphorus

Concentrations of bicarbonate-extractable organic P hydrolyzed by phosphatase enzymes ranged between 1.4 and 8.4 mg P kg<sup>-1</sup> soil, equivalent to between 37 and 87% of the bicarbonate-extractable organic P (Table 6). Of this, between 0 and 4.7 mg P kg<sup>-1</sup> soil was labile monoester P hydrolyzed by alkaline phosphatase (0–32% bicarbonate organic P) and between 1.2 and 5.8 mg P kg<sup>-1</sup> soil was total monoester P (26–77% bicarbonate organic P). Between 0 and 2.6 mg P kg<sup>-1</sup> soil was orthophosphate diesters (0–24% bicarbonate organic P). These were dominated by nucleic acids (hydrolyzed by phosphodiesterase), with only small concentrations of phospholipids. It should be noted that many of the values reported here were close to or below the limit of detection for the bicarbonate method (approximately 0.2 mg P kg<sup>-1</sup> soil).

### Relationships Between Climate, Soil Properties, and Phosphorus Fractions

#### Climate and Soil Properties

Organic C concentrations were positively correlated with concentrations of clay and oxalate Fe and Mn, and negatively correlated with soil pH (Table 7). Clay content was positively correlated with oxalate Fe and Mn, and soil pH was negatively correlated with oxalate Fe. However, clay and pH were not correlated. No soil properties were significantly correlated with CaCO<sub>3</sub> concentrations. Organic C was negatively correlated with mean annual temperature and positively correlated with mean annual precipitation when the two Texan soils (Olton and Amarillo) were omitted. Mean annual precipitation and temperature were not correlated, but a weak negative correlation existed between mean annual precipitation and soil pH.

#### Organic Phosphorus Fractions and Soil Properties

Organic P determined by NaOH-EDTA extraction and solution <sup>31</sup>P NMR spectroscopy was positively correlated with organic C, clay content, and oxalate metals (Al, Fe, Mn), and negatively correlated with soil pH (Table 8, Fig. 4). Similar relationships were evident for orthophosphate monoesters (which constituted most of the NaOH-EDTA organic P) and orthophosphate diesters, although the correlation with clay was stronger with orthophosphate diesters. Pyrophosphate was positively correlated with organic C, clay, and oxalate Mn, but was not significantly correlated with soil pH or oxalate

**Table 5.** Concentrations of functional inorganic and organic P groups in NaOH-EDTA extracts of the 18 western U.S. soils determined by solution  $^{31}\text{P}$  NMR spectroscopy. Values in parentheses are the proportion (%) of the total NaOH-EDTA extractable P.

Soil type	Inorganic orthophosphate	Pyrophosphate	Orthophosphate monoesters	Orthophosphate diesters	NMR organic P
	mg P kg <sup>-1</sup> soil				
1. Taunton	92 (90)	n.d.†	11 (10)	n.d.	11 (10)
2. Olton	51 (70)	3.1 (4.3)	19 (26)	n.d.	19 (26)
3. Declo	147 (66)	1.9 (0.9)	69 (31)	3.7 (1.7)	73 (33)
4. Warden	133 (76)	1.4 (0.8)	41 (23)	n.d.	41 (23)
5. Amarillo	83 (74)	trace	29 (26)	n.d.	29 (26)
6. Portneuf (conv subsoil)	89 (77)	2.1 (1.8)	25 (22)	n.d.	25 (22)
7. Greenleaf	167 (81)	n.d.	35 (17)	3.1 (1.5)	38 (19)
8. Williams	72 (56)	2.7 (2.1)	52 (41)	1.4 (1.1)	54 (42)
9. Portneuf (manured subsoil)	212 (83)	4.7 (1.8)	37 (15)	n.d.	37 (15)
10. Portneuf (conv)	167 (77)	3.0 (1.4)	48 (22)	n.d.	48 (22)
11. Roza	113 (74)	1.4 (0.9)	39 (25)	n.d.	39 (25)
12. Portneuf (manured)	220 (77)	1.9 (0.7)	65 (23)	n.d.	65 (23)
13. Millville	164 (73)	trace	60 (27)	n.d.	60 (27)
14. Portneuf (native)	147 (77)	2.0 (1.1)	42 (22)	n.d.	42 (22)
15. Brinegar	147 (69)	1.1 (0.5)	64 (30)	2.1 (1.0)	66 (31)
16. Palouse	165 (58)	8.3 (2.9)	107 (38)	3.3 (1.2)	111 (39)
17. Labenzo	175 (54)	13.5 (4.2)	130 (40)	2.2 (0.7)	132 (41)
18. Wahpeton	133 (49)	11.7 (4.3)	120 (44)	7.0 (2.6)	127 (47)

† n.d., not detected.

Fe. No relationships were evident between  $\text{CaCO}_3$  concentrations and NaOH-EDTA extractable organic P fractions. Soil organic P determined by ignition was positively correlated with organic C and clay, and negatively correlated with mean annual temperature, but the correlations were weaker than those for organic P

determined by NaOH-EDTA extraction and solution  $^{31}\text{P}$  NMR spectroscopy (Table 7).

Bicarbonate-extractable organic P was positively correlated with organic C, oxalate Al and Fe, and organic P extracted by NaOH-EDTA, and was negatively correlated with soil pH (Table 8, Fig. 4). All phosphatase hydrolyzable P fractions were negatively correlated with soil pH, and orthophosphate diesters were correlated with oxalate Al and Fe (Table 8). Concentrations of bicarbonate-extractable monoester and diester P were also correlated with the respective NaOH-EDTA ex-

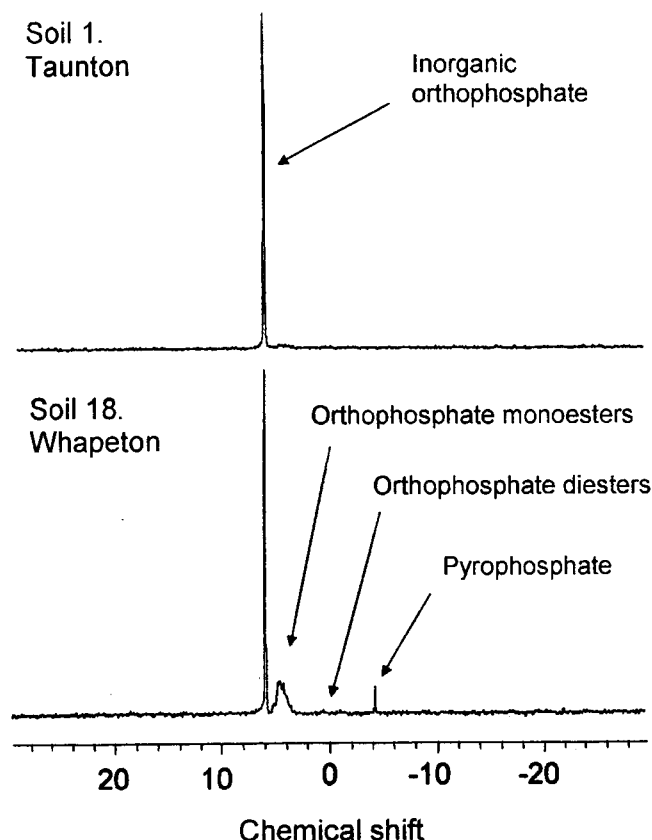
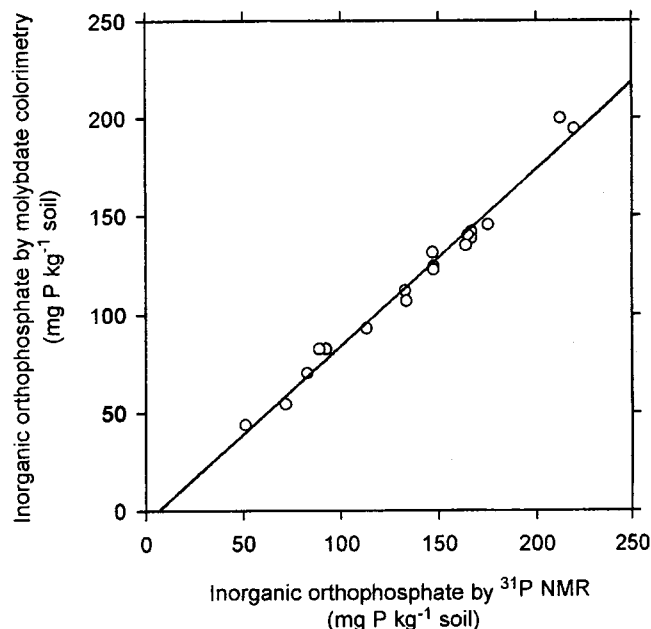
**Fig. 2.** Solution  $^{31}\text{P}$  NMR spectra of NaOH-EDTA extracts of the Taunton soil (smallest organic C concentration) and Wahpeton soil (largest organic C concentration), indicating the compounds present in the extracts.**Fig. 3.** Relationship between inorganic orthophosphate (mg P kg<sup>-1</sup> soil) in NaOH-EDTA extracts of the 18 western U.S. soils determined by molybdate colorimetry and solution  $^{31}\text{P}$  NMR spectroscopy. The regression model is described by the equation: [Orthophosphate by molybdate colorimetry] =  $0.899 \pm 0.032$ [orthophosphate by  $^{31}\text{P}$  NMR] -  $6.023 \pm 4.572$ ;  $R^2 = 0.98$ ,  $F = 815$ ,  $P < 0.0001$ ,  $n = 18$

Table 6. Concentrations of phosphatase hydrolyzable P fractions in bicarbonate extracts of 11 western U.S. soils. Values are means  $\pm$  standard deviation of triplicate extracts. Values in parentheses are the proportion (%) of the bicarbonate-extractable organic P. PMEase = alkaline phosphomonoesterase.

Soil type	PMEase (labile monoesters)		PMEase + phospholipase C (labile monoesters and phospholipids)		PMEase + phosphodiesterase (labile monoesters and nucleic acids)		Phytase (total hydrolyzed P)		Total monoesters†		Total orthophosphate diesters†	
	mg P kg <sup>-1</sup> soil		mg P kg <sup>-1</sup> soil		mg P kg <sup>-1</sup> soil		mg P kg <sup>-1</sup> soil		mg P kg <sup>-1</sup> soil		mg P kg <sup>-1</sup> soil	
1. Taunton	0.0	$\pm 0.10$ (0)	0.1	$\pm 0.10$ (3.2)	0.1	$\pm 0.17$ (5.8)	1.4	$\pm 0.32$ (81)	1.2	(72)	0.2	(9.1)
4. Warden	0.1	$\pm 0.10$ (1.5)	0.1	$\pm 0.42$ (1.5)	0.5	$\pm 0.66$ (13)	1.9	$\pm 0.42$ (50)	1.5	(39)	0.4	(12)
5. Amarillo	0.2	$\pm 0.38$ (5.4)	0.1	$\pm 0.10$ (1.4)	0.6	$\pm 0.77$ (14)	2.7	$\pm 0.26$ (66)	2.5	(61)	0.2	(4.3)
7. Greenleaf	0.5	$\pm 0.17$ (12)	0.8	$\pm 0.25$ (18)	1.2	$\pm 0.22$ (29)	2.3	$\pm 0.37$ (53)	1.3	(29)	1.0	(24)
9. Portneuf (manured subsoil)	0.2	$\pm 0.38$ (4.7)	0.2	$\pm 0.20$ (4.6)	0.3	$\pm 0.67$ (8.2)	3.8	$\pm 2.57$ (81)	3.7	(77)	0.2	(3.5)
12. Portneuf (manured)	0.5	$\pm 0.29$ (15)	0.5	$\pm 0.44$ (15)	1.1	$\pm 0.26$ (33)	2.9	$\pm 0.28$ (87)	2.3	(69)	0.6	(18)
13. Millville	1.4	$\pm 0.25$ (26)	1.4	$\pm 0.44$ (26)	1.4	$\pm 0.36$ (26)	2.6	$\pm 0.26$ (48)	2.6	(48)	0	(0)
15. Brinegar	3.4	$\pm 0.92$ (23)	3.7	$\pm 0.73$ (24)	4.8	$\pm 0.80$ (32)	6.5	$\pm 0.96$ (44)	4.8	(32)	1.7	(12)
16. Palouse	4.7	$\pm 0.77$ (21)	5.1	$\pm 0.82$ (22)	6.9	$\pm 0.59$ (30)	8.4	$\pm 0.39$ (37)	5.8	(26)	2.6	(11)
17. Lahanzo	3.6	$\pm 0.86$ (32)	3.6	$\pm 0.84$ (33)	5.4	$\pm 0.69$ (49)	6.9	$\pm 0.83$ (62)	5.0	(45)	1.9	(17)
18. Wahpeton	1.3	$\pm 0.75$ (13)	1.2	$\pm 1.17$ (12)	3.3	$\pm 0.60$ (34)	4.6	$\pm 0.86$ (47)	2.7	(27)	1.9	(20)

† Calculated as phytase hydrolyzable P minus total orthophosphate diesters.

‡ Sum of phospholipids and nucleic acids.

tractable organic P classes ( $r = 0.65$  and  $0.68$ , respectively,  $P < 0.05$ ).

### Organic Phosphorus Fractions and Climate

Organic P fractions determined by NaOH-EDTA extraction and solution  $^{31}\text{P}$  NMR spectroscopy were significantly correlated with mean annual temperature and precipitation, although in some cases the relationships were significant only when outlying soils were omitted (Table 8, Fig. 4). Mean annual temperature was negatively correlated with the concentrations and proportions of orthophosphate monoesters and diesters extracted with NaOH-EDTA, and with pyrophosphate when the two Texan soils were omitted (Table 8, Fig. 4). In contrast, mean annual precipitation was positively correlated with all NaOH-EDTA extractable organic P fractions, although again the relationships were only statistically significant for total NaOH-EDTA organic P and orthophosphate monoesters when the Texan soils were omitted.

All bicarbonate-extractable organic P fractions were negatively correlated with mean annual temperature, but only the correlation with orthophosphate diesters was statistically significant. However, the correlations with bicarbonate-extractable organic P and labile monoesters were significant when a single outlying soil (Palouse) was omitted (Table 8, Fig. 4). The proportions of labile monoesters and orthophosphate diesters in the bicarbonate extracts were also negatively correlated with mean annual temperature, but were not statistically significant. Mean annual precipitation was positively correlated with all bicarbonate-extractable organic P fractions, but was only statistically significant for bicarbonate-extractable organic P. However, the relationships were statistically significant for orthophosphate monoesters and diesters when the Texan soils were omitted.

### Regression Analysis

Soil organic P extracted by NaOH-EDTA and determined by solution  $^{31}\text{P}$  NMR spectroscopy was predicted by a model including mean annual temperature and precipitation, and a similar model described the concentration of orthophosphate monoesters (Table 9). Both models were improved slightly, but not statistically significantly, by including clay content ( $R^2 = 0.670$  and  $0.655$ , respectively). Bicarbonate-extractable organic P was predicted by a model containing soil pH and oxalate Al (Table 9). This model was improved slightly by including mean annual precipitation and temperature either alone ( $R^2 = 0.84$ ) or together ( $R^2 = 0.86$ ), but in both cases the additional variables were not statistically significant ( $P > 0.10$ ).

### DISCUSSION

Cultivated soils usually contain lower organic P concentrations than equivalent uncropped soils, because tillage increases aeration, exposing organic matter to a more vigorous microbial attack (Anderson, 1980). How-

Table 7. Correlation coefficients for relationships between physical and chemical properties of the 18 western U.S. soils.

	Organic C	pH	Clay	CaCO <sub>3</sub>	Oxalate Al	Oxalate Fe	Oxalate Mn	Mean annual temperature	Mean annual precipitation
pH	-0.57**								
Clay	0.72***	NS†							
CaCO <sub>3</sub>	NS	NS	NS						
Oxalate Al	NS	NS	NS	NS					
Oxalate Fe	0.62**	-0.75***	0.56*	NS	0.80***				
Oxalate Mn	0.69***	NS	0.83***	NS	NS	0.62**			
Mean annual temperature	-0.60**	NS	NS	NS	NS	NS	NS		
Mean annual precipitation	0.45‡	-0.52*	NS	NS	NS	NS	NS	NS	
Total P	NS	NS	NS	NS	0.47*	NS	NS	NS	-0.54*
Total inorganic P	NS	NS	NS	NS	NS	NS	NS	NS	-0.69***
Total organic P	0.80***	NS	0.57*	NS	NS	NS	NS	-0.60**	NS
Residual P	NS	-0.62**	NS	NS	0.70***	0.79***	NS	NS	NS

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

†NS, not significant.

‡  $r = 0.73^{**}$  when two outlying soils from Texas were omitted.

ever, the western U.S. soils studied here contained relatively low concentrations of organic P compared with cultivated soils from other climates and containing a wider range of soil properties (Anderson, 1980; Condrón et al., 1990a; Guggenberger et al., 1996). For example, Condrón et al. (1990a) reported organic P concentrations of 122 to 212 mg P kg<sup>-1</sup> soil in Canadian soils that had been under arable cropping for at least 70 yr.

Several interacting climate factors and soil properties are likely to contribute to the low organic matter concentrations in the soils studied here. Climate seemed to exert an important influence, as suggested elsewhere. For example, Sumann et al. (1998) reported that the proportions of orthophosphate monoesters and diesters in NaOH-NaF extracts of North American Great Plains soils correlated strongly with mean annual temperature and precipitation, while McKercher and Anderson (1968) suggested that differences in the concentrations of inositol phosphates between British and Canadian soils were linked to differences in climate. We found strong negative correlations between soil organic P concentrations and mean annual temperature. Decreasing organic P concentrations with increasing temperature was also reported for organic P in soils of South Dakota (Westin and Buntley, 1967), which probably relate to accelerated rates of microbial and enzyme activity at higher temperatures (Eid et al., 1951; Spier and Ross, 1978). The positive relationship between soil organic P and mean annual precipitation is more complex, because increases in precipitation can increase or decrease soil organic P concentrations. In wet soils, elevated soil moisture can suppress microbial activity by reducing aeration, which can lead to the accumulation of phosphonates in cool moist soils (Tate and Newman, 1982). However, in drier regions, such as much of the western USA, increasing soil moisture increases biological productivity, which in turn increases organic matter inputs to the soil (Anderson, 1980). Wetter soils also experience shorter or less intense dry periods, reducing the impact of soil drying on organic P solubility (Turner and Haygarth, 2001). The two Texan soils were consistent outliers in the relationships between soil organic P fractions and precipitation, which may reflect the combina-

tion of relatively high temperature and precipitation in that region (Table 1).

The strong correlations between organic P and C concentrations and the soil pH, clay and amorphous Fe, suggest the importance of adsorption and stabilization of organic matter in these soils. Similar correlations were reported for 168 benchmark soils from the USA with a wide range of physical and chemical properties (organic C 2–250 g C kg<sup>-1</sup> soil, pH 3.3–8.5, CaCO<sub>3</sub> 5–550 g kg<sup>-1</sup> soil) (Tiessen et al., 1984). Clays stabilize organic matter by providing reactive surfaces, which in turn provide physical protection from microbial attack. The stability of most phosphate esters increases below pH 5 (Anderson and Arlidge, 1962; Greaves and Wilson, 1969), and microbial activity is also suppressed in acidic conditions (Anderson, 1980). Thus, the soil environment exerts a strong control on organic matter accumulation. In contrast, the often substantial concentrations of CaCO<sub>3</sub> did not appear to exert a control on the accumulation of organic C or organic P compounds in these soils. Tiessen et al. (1984) reported a similar result, although Sen Gupta and Cornfield (1962) reported that organic P accumulated in calcareous soils with increasing concentrations of CaCO<sub>3</sub>.

Most of the organic P in the western U.S. soils studied here was orthophosphate monoesters, with smaller concentrations of orthophosphate diesters, which is consistent with the behavior of these functional classes of organic P in the soil. Orthophosphate monoesters are likely to be mainly inositol phosphates. These compounds constitute only small inputs to the soil in plant and microbial residues, but react strongly because of their high charge density (Turner et al., 2002b). In contrast, inputs of orthophosphate diesters to the soil are quantitatively greater than those of inositol phosphates, but their weak adsorption, especially in soils of pH greater than 5, makes them susceptible to microbial degradation (Greaves and Wilson, 1969). This differential behavior means that orthophosphate diesters are preferentially degraded, for example during the decline in soil organic P associated with long-term cultivation (Condrón et al., 1990a). Thus, orthophosphate monoesters are preferentially stabilized and accumulate to form



Table 8. Correlation coefficients for the relationships between extractable P fractions and soil physical and chemical properties. Values in parentheses are the correlation coefficients for the proportion of the total NaOH-EDTA or bicarbonate-extractable P.

	NaOH-EDTA extraction and solution <sup>31</sup> P NMR spectroscopy				Bicarbonate extraction and phosphatase hydrolysis			
	Organic P	Orthophosphate monoesters	Orthophosphate diesters	Pyrophosphate	Organic P	Labile monoester P	Total monoester P	Total orthophosphate diesters
Total P	NS†	NS	NS	NS	NS	NS	NS	NS
Total inorganic P	NS	NS	NS	NS	NS	NS	NS	NS
Total organic P	0.78***	0.79*** (0.53*)	NS	0.73***	NS	NS (0.76**)	NS	NS
Organic C	0.90***	0.90*** (0.72***)	0.67** (0.56*)	0.86*** (0.61**)	0.49*	0.62* (0.62*)	0.64*	NS
pH	-0.51*	-0.50* (-0.56*)	-0.50* (-0.48*)	NS	-0.88***	-0.80**	-0.71* (0.68*)	0.78**
Clay	0.62**	0.60** (0.50*)	0.69** (0.61**)	0.59** (0.50*)	NS	NS	NS	-0.86***
CaCO <sub>3</sub>	NS	NS	NS	NS	NS	NS	NS	NS
Oxalate Al	0.50*	0.50*	NS	NS	0.60**	NS	NS (-0.78**)	0.61*
Oxalate Fe	0.60**	0.58*	0.67** (0.63**)	NS	0.77***	NS	NS (-0.93***)	0.82*
Oxalate Mn	0.50*	0.49*	0.61** (0.54*)	0.50*	NS	NS	NS	NS
Mean annual temperature	-0.63**	-0.63** (-0.51*)	-0.54* (-0.60*)	NS‡	NS§	NS§	NS	-0.61*
Mean annual precipitation	NS‡	NS (0.57*)‡	NS	NS	0.54*	NS‡	NS‡	NS‡

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

† NS, not significant.

‡ Correlation coefficient statistically significant ( $P < 0.05$ ) when Texas soil(s) omitted.

§ Correlation coefficient statistically significant ( $P < 0.05$ ) when Palouse soil omitted.

the dominant class of organic P in most soils, including the western U.S. soils of the current study.

Pyrophosphate was also present in most soils and appeared to be controlled by similar mechanisms to both orthophosphate monoesters and diesters. Inorganic polyphosphates appear to originate from microbial activity and are rapidly degraded in soils, but pyrophosphate (an inorganic polyphosphate with chain length  $n = 2$ ) can become stabilized in soils and remain for many months (Blanchar and Hossner, 1969). Therefore, it is unclear whether the presence of pyrophosphate reflects microbial activity or the potential for stabilization in the soil.

Concentrations of bicarbonate-extractable organic P were controlled primarily by soil pH rather than climate or clay content. A similar strong relationship was reported for a range of UK pasture soils, in which bicarbonate-extractable organic P concentrations increased markedly in soils less than pH 5 (Turner and Haygarth, 2003). Bicarbonate-extractable organic P has been suggested to be an active pool of soil organic P, and probably represents compounds that are readily exchangeable from the soil (Bowman and Cole, 1978). However, there is little information on the chemical composition of bicarbonate-extractable organic P with which to infer its biological availability. In the current study, bicarbonate-extractable organic P was of a similar composition to the total soil organic P determined by NaOH-EDTA extraction and <sup>31</sup>P NMR spectroscopy, which agrees with the organic P composition of Canadian arable soils determined using solution <sup>31</sup>P NMR analysis of bicarbonate and NaOH extracts (Zhang et al., 1999). The main difference between the composition of bicarbonate and NaOH-EDTA extractable organic P in the western U.S. soils was the greater proportions of diesters in bicarbonate extracts. This probably reflects the greater sensitivity of the phosphatase hydrolysis technique, which can detect lower concentrations of orthophosphate diesters than solution <sup>31</sup>P NMR spectroscopy, plus the rapid degradation of some orthophosphate diesters (RNA and some phospholipids) in alkaline solution, which will lead to the underestimation of orthophosphate diesters in most soils (Turner et al., 2003).

Bicarbonate-extractable organic P concentrations in these western U.S. soils were low, but large proportions were hydrolyzable by common soil phosphatase enzymes and were, therefore, potentially bioavailable. Similar results were reported for volcanic soils of the Canary Islands, in which between 33 and 93% of the bicarbonate-extractable organic P was hydrolyzed by a nonspecific acid phosphatase from wheat (Negrin et al., 1995). These large proportions of phosphatase-hydrolyzable organic P may partly explain discrepancies in correlations between plant-available P pools and crop response, and support the suggestion that soil organic P could account for the difficulties encountered in determining soil P fertility using inorganic P determinations alone (Tieszen et al., 1984). Our results should be considered with caution, however, because analytical error was large relative to the small concentrations of phosphatase hydrolyzable P in these soils. Further, the

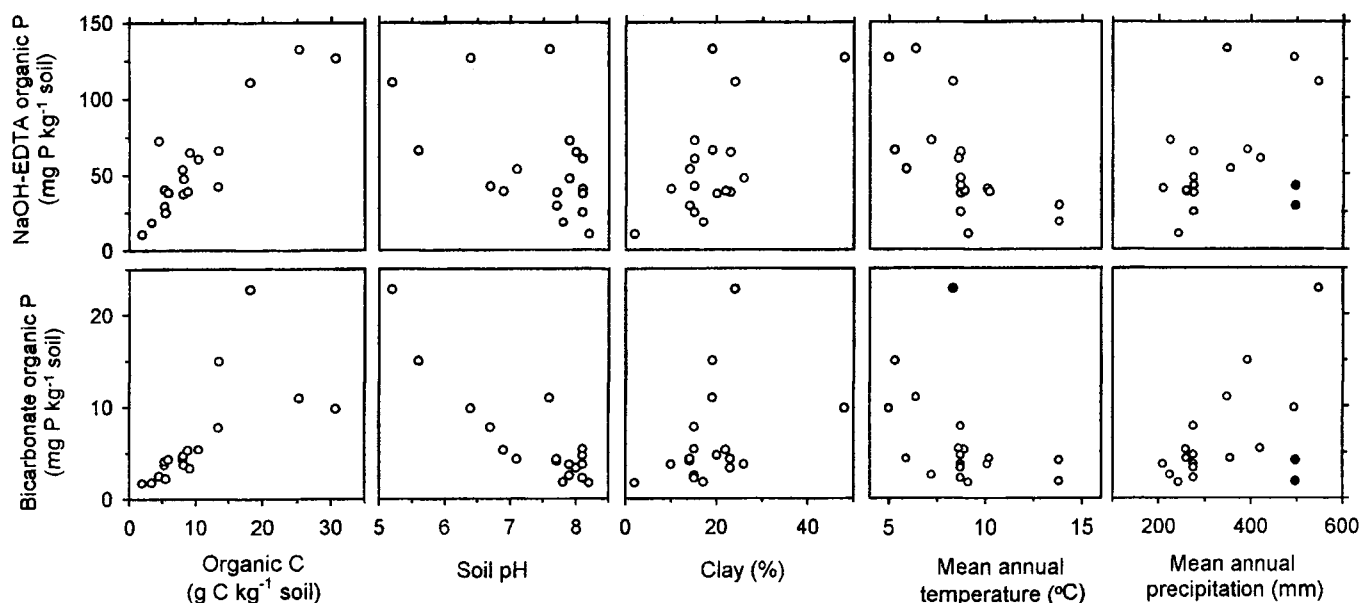


Fig. 4. Relationships between soil properties, climate variables (mean annual precipitation [mm] and temperature [°C]) and organic P determined by bicarbonate extraction and molybdate colorimetry, and by NaOH-EDTA extraction and solution  $^{31}\text{P}$  NMR spectroscopy ( $\text{mg P kg}^{-1}$  soil). Outlying soils not included in the calculation of correlation coefficients are identified as shaded circles.

results are in direct contrast to studies of Japanese and Australian arable soils, in which only small proportions of bicarbonate-extractable organic P were hydrolyzed by phosphatase and phytase (Otani and Ae, 1999; Hayes et al., 2000). Bicarbonate-extractable organic P concentrations can also vary considerably in response to soil drying (Turner and Haygarth, 2003), so the results for air-dried soils may not readily translate to field conditions. However, the soils studied here are dry for much of the year, and regularly experience temperatures at the surface in excess of  $50^\circ\text{C}$  during the summer, suggesting that mild drying is unlikely to greatly influence organic P solubility.

Conventional techniques for measuring total soil organic P suffer from various errors that can severely limit their accuracy (Condon et al., 1990b). The ignition method overestimates organic P in most soils because high temperature ignition alters the solubility of mineral P compounds (Williams et al., 1970). This in turn limits confidence in extraction techniques, because there is no means of quantifying the recovery of organic P (Bowman and Moir, 1993). The current study highlights a further source of error in the measurement of extracted organic P by colorimetry, because inorganic orthophosphate in alkaline extracts is underestimated by molybdate reaction. This has been noted elsewhere (Bedrock et al., 1994; Hupfer and Gächter, 1995; Guggenberger

et al., 1996) and probably indicates the presence of orthophosphate-metal-humic complexes not detected by molybdate reaction. In the current study, organic P concentrations determined by NaOH-EDTA extraction and solution  $^{31}\text{P}$  NMR spectroscopy appeared closer to the likely true values than those determined by ignition, because extracted organic P was more strongly correlated with organic C, and gave slightly greater organic C/organic P ratios (Condon et al., 1990b; Bowman and Moir, 1993). The degradation of RNA and phospholipids in NaOH-EDTA does not affect the determination of total organic P determined by NaOH-EDTA extraction and solution  $^{31}\text{P}$  NMR spectroscopy, because these compounds degrade to orthophosphate monoesters rather than inorganic orthophosphate (Turner et al., 2003). Taken together, these results suggest that NaOH-EDTA extraction and solution  $^{31}\text{P}$  NMR spectroscopy provides the most accurate estimate of soil organic P.

## CONCLUSIONS

The organic P extractable by NaOH-EDTA in irrigated agricultural soils of the western USA with a wide range of textural and chemical properties was mainly orthophosphate monoesters, with smaller concentrations of orthophosphate diesters and pyrophosphate.

Table 9. Regression models describing the influence of climate and soil properties on (1) soil organic P determined by NaOH-EDTA extraction and solution  $^{31}\text{P}$  NMR spectroscopy, (2) orthophosphate monoesters by the same technique, and (3) bicarbonate-extractable organic P.

Y variate	$X_1$ variate	Gradient $X_1$	$X_2$ variate	Gradient $X_2$	Intercept	$R^2$	F value	P value
1. NaOH-EDTA organic P, $\text{mg P kg}^{-1}$ soil	Mean annual temperature, $^\circ\text{C}$	$-10.1 \pm 2.33$	Mean annual precipitation, mm	$0.15 \pm 0.05$	$91.8 \pm 25.7$	0.622	12.3	0.0007
2. NaOH-EDTA orthophosphate monoesters, $\text{mg P kg}^{-1}$ soil	Mean annual temperature, $^\circ\text{C}$	$-9.60 \pm 2.26$	Mean annual precipitation, mm	$0.15 \pm 0.05$	$88.9 \pm 25.0$	0.612	11.8	0.0008
3. Bicarbonate organic P, $\text{mg P kg}^{-1}$ soil	Soil pH	$-4.58 \pm 0.71$	Oxalate Al, $\text{g Al kg}^{-1}$ soil	$5.99 \pm 2.69$	$36.3 \pm 6.25$	0.829	36.3	<0.0001

Phosphatase hydrolysis of bicarbonate-extractable organic P revealed a similar composition, and suggested the potential biological availability of this readily exchangeable organic P pool. Strong correlations between soil organic P, climate, and soil properties suggested that equilibrium levels of organic P in western U.S. soils reflect a balance between the physical protection offered by the soil matrix, and the suitability of the environment for biological productivity. In contrast, the presence of often large concentrations of  $\text{CaCO}_3$  appeared to have little influence on soil organic P concentrations.

### ACKNOWLEDGMENTS

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